906-Pos Board B675

Visualizing the Formation and Exploring the Structure and Dynamics of DNA-Architectures. A Single Molecule Study

Amani A. Hariri, Graham Hamblin, Hanadi Sleiman, Gonzalo Cosa.
McGill University, Montreal, QC, Canada.

DNA nanotubes are promising materials with potential applications in medicine, nanotechnology and biotechnology fields. Their unique versatility is the result of their high aspect ratio and encapsulation potential, rigidity, relative ease of preparation and biocompatibility.[1] Our goal is to couple the DNA nanotubes-assembly with a controllable surface patterning in order to generate robust, programmable and functional scaffolds for the assembly of other species such as proteins, cells, etc. These materials would play an increasingly important role in a diverse range of applications, such as drug delivery, diagnostics, tissue engineering, 'smart' optical systems and biosensing. In order to obtain higher quality materials, control on their formation is highly desirable. We have envisioned that single molecule spectroscopy provides the right tool set to tackle the formation kinetics, structural and dynamic studies of these novel nanostructures. We are currently involved in investigating, via single molecule spectroscopy methodologies, the assembly dynamics of DNA nanotubes. Here, we have demonstrated a step by step bottom-up assembly of DNA nanotubes by following their growth on the surface after adding sequentially Cy3-labbeled DNA-rungs and linkers: This was confirmed by single-molecule photobleaching analysis. I will describe in this presentation the details of our studies and the structural and dynamic information gained on the new DNA-structures. Our studies constitute a step towards achieving a higher-order control of the assembly of the nanostructures.

1. (a) Aldaye, F. A.; Lo, P. K.; McLaughlin, C. K.; Karam, P.; Cosa, G.; Sleiman, H. F.; Nature Nanotech. 2009, 4, 349-352. (b) Lo, P.K.; Karam, P.; Aldaye, F.A.; McLaughlin, C.K.; Hamblin, G.D.; Cosa, G.; Sleiman, H.F. Nature Chemistry, 2010, 2, 319-328. (c) Lo, P.K.; Altvater, F.; Sleiman, H.F. J. Am. Chem. Soc., 2010, 132, 10212-10214.

907-Pos Board B676

Single-Stranded DNA Scanning and Deamination by APOBEC3G with Single-Molecule Resolution

Gayan Senavirathne[1], Malgorzata Jaszczur[2], Paul A. Auerbach[2], Thomas G. Upton[2], Linda Chelico[2], Myron F. Goodman[2], David Rueda[1].
[1]Department of Chemistry Wayne State University, Detroit, MI, USA, [2]Departments of Biological Sciences and Chemistry, University of Southern California, Los Angeles, CA, USA.

APOBEC3G (Apo3G) is a deoxycytidine deaminase that converts C → U on single-stranded (ss) DNA, favoring ataCCCaaa and disfavoring tttCCCttt motifs. Apo3G inactivates HIV-1 by attacking cDNA in T cells lacking the viral infectivity factor (Vif). Here, we observe Apo3G scanning and C → U product formation in real-time on ssDNA using single-molecule fluorescence. Apo3G displays short (<25 s) and long duration (25 s - 10 min) binding events about equally distributed. Mean displacements from the initial binding site are about 10 Å, which vary depending on motif location and identity. Apo3G "long binders" retain contact with the DNA, but do not remain tethered at a single binding site. Instead, the time trajectories suggest a random, bidirectional motion, while contracting the ssDNA in a motif-dependent manner. Our data further indicate that the enzyme tends to scan more often in the vicinity of a hot motif, especially when located near the 5′-end. Using Cy5-labeled Pfu DNA polymerase, which binds U on ssDNA selectively, we show that Apo3G-catalyzed deaminations are strongly favored in the 5′-direction. The observation that random scanning generates non-random catalysis supports a model in which Apo3G binds in an asymmetric catalytically active orientation.

908-Pos Board B677

Single Particle Tracking to Characterize the Mechanism of Pore Formation by EquinatoxinII

Yamunadevi Subburaj, Ana J. García Sáez.
Max-Planck Institute for Intelligent systems, Heidelberg, Germany.

Equinatoxin II (EqtII) is a pore forming protein of the actinoporin family. The activity of EqtII depends on the presence of sphingomyelin in the target membrane. The mechanism of pore formation by EqtII involves several steps such as, i) secretion of unfolded polypeptide and folding into a water-soluble protein, ii) Binding of water soluble monomer to the target membrane and iii) subsequent oligomerization of several monomers on the surface of the membrane leading to the formation of a functional pore. Despite intense research, the structural and dynamic aspects of oligomerization and membrane permeabilization by EqtII remains poorly understood. In this study, we have investigated the dynamics, oligomerization and pore forming process of EquinatoxinII, using single color particle tracking with total internal reflection microscopy. Our results show that the number of trimer and tetramer increases over time, whereas the number of dimers stays more or less constant. The level of monomers fluctuate which may be due to the binding of new proteins from the solution. We observed two main types of diffusion, a) about 10 % of the population shows Brownian motion with diffusion coefficient $D=0.60 \pm 0.01$ μm2/s and b) 90% shows confined diffusion with the diffusion coefficient $D=0.36 \pm 0.03$μm2/s. We propose that either trimer or tetramer could be responsible for the formation of a functional pore.

909-Pos Board B678

Counting Membrane Proteins in Liposomes for Single-Molecule Microscopy

Janice Robertson[1], Larry Friedman[1], Ludmila Kolmakova-Partensky[2], Mike Rigney[2], Jeff Gelles[1], Christopher Miller[2].
[1]Brandeis University, Waltham, MA, USA, [2]HHMI/Brandeis University, Waltham, MA, USA.

We can learn a lot by studying membrane proteins using single-molecule techniques. For example, electrical recordings of single ion channels have provided a wealth of information about key functional states. To obtain structural information, fluorescence microscopy can be used to monitor single-molecule Forster resonance energy transfer revealing dynamic conformational details. Experiments of this sort are being done but usually examine the protein in detergent micelles rather than a lipid environment. However, for many questions, studying the protein in lipid membranes is essential. While it is possible to carry out microscopy on proteins in supported membranes, formation of the bilayer can be complicated and often limits the lipid conditions. Another method is to simply examine the protein in liposomes, the benefits being that it is quick, easy and reconstitution is robust under different lipid compositions. In order to prepare this approach for single-molecule studies, we must establish the conditions in which we have a single protein molecule per liposome. Reconstitution statistics follows a Poisson distribution, which can be measured as a function of protein density and liposome size. Here we measure the size distributions of liposomes formed with E. Coli polar lipids and those containing mixtures of phosphatidyl-ethanolamine (PE), -glycerol (PG) and -choline (PC), by cryo-electron microscopy and fluorescence intensity of Alexa-488 labelled PE by total internal reflection fluorescence microscopy. The protein occupancy is measured as a function of protein density by photo-bleaching analysis of the Cl-/H+ transporter CLC-ec1 labelled with Cyanine-5. The results provide a foundation for future single-molecule studies of membrane proteins, such as the identification of conformational changes as well as the determination of protein stoichiometry with respect to studying the thermodynamics of protein association in membranes.

910-Pos Board B679

A Bayesian Approach to Single Particle Tracking Analysis

Fredrik Persson[1], Martin Lindén[2], Cecilia Unosson[1], Johan Elf[1].
[1]Uppsala University, Uppsala, Sweden, [2]Stockholm University, Stockholm, Sweden.

Single particle tracking using photoactivatable fluorescent proteins (spt-PALM) has been shown capable of following the movements of individual proteins in living cells. It is also possible to classify individual trajectories into coarsely predefined states of diffusion, corresponding for example to different states of binding. This suggests that it should be possible to determine intracellular state transition rates under steady state conditions simply by determining the average time an individual molecule spends in one state. In this way the single molecule nature of spt-PALM makes it possible to overcome the averaging that normally masks all information about transition rates in a steady state ensemble.

Two major challenges have to be overcome in order to study kinetics by tracking individual cytoplasmic proteins. First, there has been no objective way to determine the number of diffusion states based on experimental data alone. Instead a number of states are commonly presumed, e.g. two states, bound and free, and the data is fitted under these assumptions. Second, intracellular single molecule diffusion trajectories from fluorescent fusion proteins are typically very short. The challenge is therefore to correctly combine the information from thousands of short trajectories (<20 steps) instead of a few very long trajectories with multiple state transitions.

We provide an analytical tool based on a Variational Bayesian treatment of a Hidden Markov Model, to interpret the motion and binding kinetics of fluorescently tagged proteins in the cytoplasm. As opposed to previously used HMM for diffusive particles, our VB-HMM method is capable of learning the number of states as well as transition rates between these states from the data. This is achieved by using a single model to describe all the typically short trajectories obtained when tracking single molecules in the cytoplasm.